Annals of Botany **98**: 351–360, 2006
doi:10.1093/aob/mcl105, available online at www.aob.oxfordjournals.org

Inbreeding Depression and Mixed Mating in *Leptosiphon jepsonii*: A Comparison of Three Populations

CAROL GOODWILLIE* and MARY CATHERINE KNIGHT

Department of Biology, East Carolina University, Howell Science Complex, Greenville, NC 27858, USA

Received: 21 December 2005 Returned for revision: 24 February 2006 Accepted: 24 March 2006 Published electronically: 23 May 2006

• *Background and Aims* Inbreeding depression is thought to play a central role in the evolution and maintenance of cross-fertilization. Theory indicates that inbreeding depression can be purged with self-fertilization, resulting in positive feedback for the selection of selfing. Variation among populations of *Leptosiphon jepsonii* in the timing and rate of self-fertilization provides an opportunity to study the evolution of inbreeding depression and mating systems. In addition, the hypothesis that differences in inbreeding depression for male and female fitness can stabilize mixed mating in *L. jepsonii* is tested.
• *Methods* In a growth room experiment, inbreeding depression was measured in three populations with mean outcrossing rates ranging from 0·06 to 0·69. The performance of selfed and outcrossed progeny is compared at five life history stages. To distinguish between self-incompatibility and early inbreeding depression, aborted seeds and unfertilized ovules were counted in selfed and outcrossed fruits. In one population, pollen and ovule production was quantified to estimate inbreeding depression for male and female fitness.
• *Key Results* Both prezygotic barriers and inbreeding depression limited self seed set in the most outcrossing population. Cumulative inbreeding depression ranged from 0·297 to 0·501, with the lowest value found in the most selfing population. Significant inbreeding depression for early life stages was found only in the more outcrossing populations. Inbreeding depression was not significant for pollen or ovule production.
• *Conclusions* The results provide modest support for the hypothesized relationship between inbreeding depression and mating systems. The absence of early inbreeding depression in the more selfing populations is consistent with theory on purging. Differences in male and female expression of inbreeding depression do not appear to stabilize mixed mating in *L. jepsonii*. The current estimates of inbreeding depression for *L. jepsonii* differ from those of previous studies, underscoring the effects of environmental variation on its expression.

Key words: Inbreeding depression, *Leptosiphon jepsonii*, male fitness, mating system, mixed mating, Polemoniaceae, self-incompatibility.

INTRODUCTION

Inbreeding depression, the reduced fitness of inbred relative to outbred progeny, is thought to play a central selective role in the origin and maintenance of cross-fertilization in higher plants (Darwin, 1877; Charlesworth and Charlesworth, 1987). Most plants are hermaphroditic and, therefore, have the potential for self-fertilization. Self-fertilization is prevented in many species by genetic self-incompatibility (SI) systems (de Nettancourt, 1977) or floral traits that prevent self-pollen deposition (Richards, 1997). The extent of self-fertilization (selfing) versus cross-fertilization (outcrossing) varies widely among plant species. A selfing allele in an outcrossing population is expected to have an inherent genetic transmission advantage because it is passed along in selfed seeds through both pollen and ovules, whereas the seeds of outcrossing plants carry only one copy of their genes (Fisher, 1941). When pollen discounting is negligible (i.e. when selfing does not reduce male outcross success), a >50 % reduction in the fitness of selfed progeny is required to outweigh the transmission advantage of selfing and favour outcrossing (Charlesworth and Charlesworth, 1979; Holsinger *et al*., 1984).

The evolutionary dynamics of mating systems are complicated, however, by the fact that inbreeding depression can evolve with the selfing rate. Theory indicates that, with self-fertilization, inbreeding depression caused by deleterious recessive alleles can be purged by selection (Lande and Schemske, 1985). This finding prompted Lande and Schemske (1985) to argue that only the two mating-system extremes—selfing with low inbreeding depression and complete outcrossing with high inbreeding depression—should be evolutionarily stable. Purging poses an impediment to the stability of mixed mating systems (plants that reproduce by both selfing and outcrossing) because it creates positive selective feedback; as selfing increases, the major disadvantage of selfing decreases (Lande and Schemske, 1985).

Mixed mating systems do exist in a substantial proportion of higher plant species, however (Goodwillie *et al.*, 2005), and subsequent models have identified factors that can, in theory, lead to their stability, including biparental inbreeding, pollen discounting and resource allocation (reviewed in Goodwillie *et al.*, 2005). However, many of these models treat inbreeding depression as a constant and do not take into account its evolution with the selfing rate. Therefore, in the continuing pursuit of evolutionary explanations for mixed mating, there is a need to understand the extent to which purging is a potent

* For crrespondence. E-mail goodwilliec@mail.ecu.edu

evolutionary force. Despite considerable empirical investigation, the extent and patterns of purging in real populations remain equivocal (Crnokrak and Barrett, 2002), as does the evolutionary stability of mixed mating systems.

Comparisons of closely related plant species with different rates of self-fertilization have provided insight into the evolutionary dynamics of inbreeding depression (Holtsford and Ellstrand, 1990; Carr and Dudash, 1996; Johnston and Schoen, 1996; reviewed in Byers and Waller, 1999). A meta-analysis of these comparative studies found that purging was inconsistent across plant taxa (Byers and Waller, 1999). Of studies published since that analysis, most have added empirical support for the purging hypothesis (Cheptou *et al*., 2000; Fishman 2001; Busch, 2005; Mustajarvi *et al*., 2005) but at least one has yielded equivocal results (Affre and Thompson, 1999). *Leptosiphon* (formerly *Linanthus*), a genus of annual plants in the Polemoniaceae, provides an ideal system for such an approach. The genus contains both strongly SI and self-compatible (SC) species (Grant and Grant, 1965; Goodwillie, 1999). A previous study compared the magnitude of inbreeding depression in *L. bicolor*, a highly selfing species to that of its partially outcrossing sister species, *L. jepsonii* (Goodwillie, 2000). Although inbreeding depression was low in both species, estimates for the more outcrossing species were consistently higher, supporting the purging hypothesis.

Since that study, an unusual floral age-dependent mechanism in which stigmas are initially SI and become SC after 24–48 h has been discovered in *L. jepsonii* (Goodwillie *et al.*, 2004). A similar phenomenon has been reported in *Campanula rapunculoides* (Stephenson *et al*., 1992; Vogler and Stephenson, 2001), and both appear to confer delayed self-fertilization. Delayed selfing mechanisms allow outcrossing when visitation occurs, but can provide assurance of reproduction through selfing when pollinators or mates are scarce (Lloyd, 1979, 1992; Kalisz and Vogler, 2003). In some individuals of *L. jepsonii*, however, stigmas are fully SC when flowers first open, which promotes earlier and a potentially higher rate of selfing. In a previous study, the frequency of early SC phenotypes in populations was found to be negatively related to the outcrossing rate (Goodwillie and Ness, 2005). Substantial variation among populations of *L. jepsonii* in the timing of SC, outcrossing rates and floral size traits (Goodwillie and Ness, 2005) provides an opportunity for intraspecific comparisons.

As part of a broad investigation of evolution in the mating system of *L. jepsonii*, it is questioned whether the magnitude of inbreeding depression differs among three populations with contrasting mating systems, and its role in the maintenance of partial outcrossing is considered. The finding of partial SI in *L. jepsonii* motivates a detailed exploration of the factors that limit seed set from self-pollination. Here ovule fertilization and abortion in selfed and outcrossed fruits are quantified to distinguish between the effects of prezygotic mechanisms, such as SI, versus post-zygotic processes, such as inbreeding depression. Finally, inbreeding depression in pollen and ovule production for one population of *L. jepsonii* is investigated. Theory indicates that differential expression of inbreeding depression in male and female function could stabilize mixed mating (Rausher and Chang, 1999). In only a handful of studies, however, has inbreeding depression been estimated for separate measures of male and female fitness. Experimental investigations of this kind are needed to assess whether differences in male and female inbreeding depression play a role in maintaining mixed mating systems.

TABLE 1. *Mating systems, timing of SC and floral traits of study populations*

	LH	WR	IC
Mean outcrossing rate (*t*)	0·06	0·37	0·69
Distribution of SI types			
Flowers always SC (%)	71	16	10
Flowers SI 1 day (%)	23	78	30
Flowers SI 2 days (%)	3	3	35
Flowers SI ⩾3 days (%)	3	3	25
Mean corolla lobe length (mm)	3·8	4·3	4·2
Mean corolla tube length (mm)	27·7	32·7	39·9

Mean outcrossing rates were calculated from allozyme analysis of progeny arrays in 2003 and 2004 for LH and IC and 1996, 2003 and 2004 for WR.

For distribution of SI types, plants sampled from populations were assigned to one of four classes based on the number of days that flowers remained SI. Sample sizes for SI determination and floral measurements ranged from 29 to 32.

All data are from Goodwillie (2000) or Goodwillie and Ness (2005).

MATERIALS AND METHODS

Species and study populations

Leptosiphon jepsonii is a spring annual with a restricted distribution in the North Coast Ranges of California (Schemske and Goodwillie, 1996). Cross-pollination is effected by beeflies, but substantial autonomous self-pollination has been observed in greenhouse studies (Goodwillie and Ness, 2005). Study populations near Lake Hennessey and at the Wantrup Reserve (both in Napa County) and a population on Ida Clayton Road (Sonoma County) were chosen to span the range of variation in outcrossing rates, floral morphology and frequency of individuals with early versus late SC (Table 1), as previously reported (populations 6, 16 and 7, respectively, in Goodwillie and Ness, 2005). All three populations occur on grassy meadow openings in oak woodlands. The population on Ida Clayton Road extends onto an isolated patch of serpentine soil. Population sizes for Lake Hennessey (LH), Wantrup Reserve (WR) and Ida Clayton Road (IC) were estimated in 2004 as 30 000, 16 000 and 50 000, respectively.

Experimental design

In December 2004 and January 2005, plants were raised from field-collected seeds in an indoor growth room with

natural and artificial lighting. Of these, ten plants from each study population were selected randomly to serve as maternal plants and were assayed for the timing of SC using methods described in a previous study (Goodwillie *et al*., 2004). To investigate inbreeding depression, flowers were emasculated as buds and hand-pollinated with self or outcross pollen. Cross-pollinated flowers were brushed with anthers from three plants of the same population. Approximately 20–30 pollinations of each cross-type were performed on each plant, and sepals were marked to indicate pollination treatment in developing fruits.

To examine the effects of pre- and post-zygotic processes that limit self-seed set, some fruits were dissected approx. 1 week prior to maturation. A distinction was made between ovules that were (*a*) fertilized and maturing normally, (*b*) fertilized and aborted and (*c*) unfertilized. Normally maturing seeds were green, round and enlarged. Ovules that were white in colour, enlarged and flattened or abnormally shaped were scored as fertilized and aborted. Unfertilized ovules were green and could be distinguished from fertilized seeds by their considerably smaller size (Levin, 1989). Five fruits of each cross-type were dissected from each maternal plant. The remaining fruits were allowed to mature and then harvested.

Of the seeds harvested, approx. 50 of each maternal plant and cross-type were placed on moist filter paper at 4 °C to initiate germination. The germination rate was scored as the proportion of seeds that had germinated after 4 weeks. Thirty to forty germinated seeds were planted per maternal plant and cross-type for each population. In a few plants, low germination rates limited seedling numbers to <30 (mean $n = 32{\cdot}6$). Seedlings were planted in standard potting medium in 'Conetainers' (Stuewe and Sons, Corvallis, OR, USA), placed in a controlled growth chamber and top-watered daily. At the end of 2 weeks, all seedlings were scored for survival.

From the surviving seedlings, 12 of each maternal plant and cross-type were chosen randomly for inclusion in the remainder of the experiment and moved to the growth room. In some maternal plant/cross-types, final sample sizes were <12 because of low germination or seedling survival, yielding a final total sample size of 680 plants (mean $n = 11{\cdot}3$, minimum = 10). Plants were distributed among 12 groups of racks, each containing one replicate of each maternal plant/cross-type per population. These groups were rotated around the growth room once per week to minimize experimental error caused by environmental variation. Plants were top-watered daily and fertilized weekly with a mild solution of 12–55–6 commercial fertilizer. Plants were inspected daily and the initiation of flowering recorded. After senescence, plants were cut at soil level, dried and weighed. In a previous study of *L. jepsonii*, above-ground biomass was shown to be strongly correlated with flower production (Goodwillie, 2000).

For the WR population only, inbreeding depression for additional components of female fitness (ovules per flower) and male fitness (pollen number per flower) was measured. Ovules were counted in three flowers in a random subset of four plants of each family and cross-type. From all experimental plants that produced flowers, anthers from three newly opened flowers were collected and pooled and then placed in 1% saline solution. Pollen grains were counted using a haemocytometer, drawing 12 samples from the pooled pollen to estimate the number of grains per flower for each plant. A distinction was made between two pollen size classes during counting. Studies of other species (Kelly *et al*., 2002) indicate that small pollen is likely to have lower viability.

Statistical analyses

All statistical analyses were performed on SPSS version 13·0 (SPSS, 2005). Analysis of variance was used to test treatment effects for each life stage. Because the study populations were chosen to represent contrasting levels of outcrossing, population was treated as a fixed factor in all analyses. The full model used for seed set variables and above-ground biomass included populations (fixed factor), cross-treatments (selfed or outcrossed, fixed factor) and maternal plants (random factor nested within populations), with fruits or progeny as the unit of replication. A significant interaction between population and cross-treatment rejects the null hypothesis that the magnitude of inbreeding depression does not differ among populations. Variation among families in inbreeding depression is indicated by a significant interaction between maternal plant and cross-treatment. For germination and survival rates, data for each maternal plant and cross-type were unreplicated; therefore, it was not possible to test for among-family variation. To improve homogeneity of variances, above-ground biomass was log transformed, and the proportion of fertilized ovules forming seeds, rate of germination and rate of survival to flowering were arcsine transformed. Johnston and Schoen (1994) note that, to test for variation among families or populations in inbreeding depression, data should be log transformed so that differences between cross-treatments are converted to ratios. For some of the variables, however, log transformation resulted in data that violated model assumptions and, therefore, was not used. In only one case did a log transformation produce a qualitatively different result (see Results).

Analysis of variance for all life stages was also carried out separately for each population, with cross-treatment and maternal plant as factors. It was possible to test for variation among families in inbreeding depression (treatment × maternal plant interaction) in only seed set and biomass variables, for which there was replication within maternal plants. The same model was used for analysis of variance of pollen and ovule number (both log transformed) in the WR population.

Inbreeding depression (δ) was calculated for each life stage and population as $\delta = 1 - (w_s/w_o)$, where w_s and w_o are the fitness of selfed and outcrossed progeny, respectively. Cumulative inbreeding depression was calculated

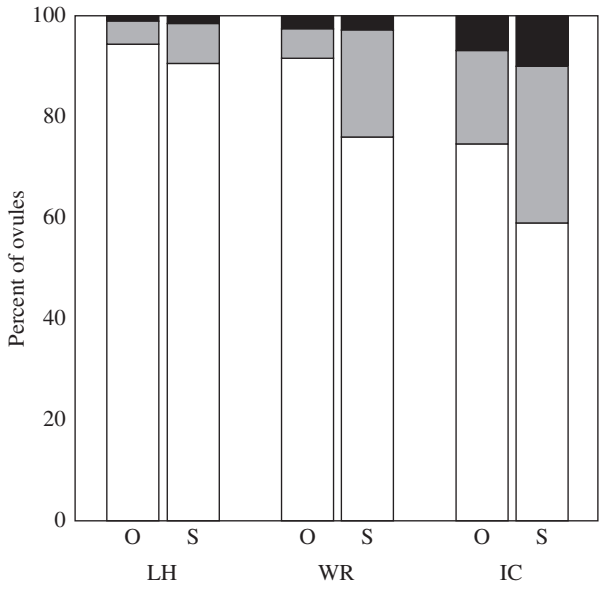

FIG. 1. Fate of ovules in hand outcross- and self-pollinated flowers. Open bars indicate normal seeds; shaded bars indicate fertilized and aborted ovules; black bars indicate unfertilized ovules. O, Outcrossed flowers, S, selfed flowers. Percentages are calculated across flowers of all maternal plants per population.

by multiplying fitness values for each cross-type across all life stages and then applying the formula above.

RESULTS

Seed set

In IC, the most outcrossing population, prezygotic mechanisms incur a marginally significant reduction in selfed seed set, with 9·1 % of ovules remaining unfertilized after self-pollination versus 6·2 % unfertilized with outcross pollination (d.f. = 1, $F = 4{\cdot}069$, $P = 0{\cdot}065$; Fig. 1). In the more selfing LH and WR populations, the proportion of unfertilized ovules was low overall and not significantly different in selfed and outcrossed fruits. The rate of fertilization was not affected by cross-treatment in the overall ANOVA (d.f. = 1, $F = 1{\cdot}19$, $P = 0{\cdot}282$), but the population effect was significant (d.f. = 2, $F = 7{\cdot}18$, $P = 0{\cdot}002$) as was the interaction between population and cross-treatment (d.f. = 2, $F = 3{\cdot}23$, $P = 0{\cdot}05$), indicating a significant difference among populations in the strength of prezygotic barriers to self-fertilization.

Significant inbreeding depression was found for the proportion of fertilized ovules that developed into seeds only in the more outcrossing WR and IC populations (Table 2). In the overall ANOVA, populations differed in the magnitude of inbreeding depression at this stage, as indicated by a significant interaction between population and cross-treatment (Table 3). Inbreeding depression was highest in IC and lowest in the LH population (Table 4 and Fig. 1).

Germination and survival

In no population was there significant inbreeding depression for germination rate (Table 2). Mean germination rates for both cross-treatments were high for the LH and WR populations and significantly lower for IC seeds (Tables 3 and 4). Populations did not differ significantly in the relative germination rate of selfed and outcrossed seeds (Table 3).

Inbreeding depression for seedling survival to 2 weeks was observed in the IC population, but not in the more selfing LH or WR populations (Table 2). Accordingly, the overall ANOVA revealed a significant interaction between population and treatment (Table 3); however, this interaction was not significant when data were log transformed as per Johnston and Schoen (1994). Survival from 2 weeks to flowering was significantly affected by cross-treatment in only the IC population (Table 2). Inbreeding depression for survival to flowering did not differ significantly among populations, however (Table 3).

Biomass

Above-ground biomass was significantly lower in selfed than outcrossed progeny in the overall ANOVA and the interaction between population and treatment was not significant (Table 3). At the level of populations, however, only the intermediately outcrossing WR population showed a significant effect of cross-treatment. Significant variation among families in the magnitude of inbreeding depression was found in both the overall ANOVA and in population analyses for LH and WR. Error variation for above-ground biomass was substantial, with values often ranging more than an order of magnitude for replicates within a family and cross-treatment. For example, among selfed progeny of family 10 in the LH population, biomass ranged from 0·04 to 1·76 g.

Cumulative inbreeding depression for seed set, germination, seedling survival, survival to flowering and biomass was 0·297, 0·495 and 0·501 in the LH, WR and IC populations, respectively.

Ovule and pollen number in the WR population

Ovule number was not significantly different in selfed and outcrossed progeny, nor did maternal families vary significantly (Table 5). The interaction between maternal family and pollination treatment was significant, however, indicating that the difference between selfed and outcrossed progeny for ovule number varied significantly among families. Analysis of variance found no significant effect of cross-treatment on the number of large pollen grains produced (Table 5); however, the value for inbreeding depression for this component of fitness was substantial (Table 4). The proportion of small (and presumably nonviable) pollen grains varied significantly among families but was not related to inbreeding depression, as neither the cross-treatment effect nor its interaction with maternal plants were significant. Including all shared life stages, cumulative inbreeding depression was 0·503 for female fitness and 0·571 for male fitness.

TABLE 2. *Analysis of variance of the effects of treatment (self- versus cross-fertilization, fixed factor) and maternal plant (random factor) for individual populations*

Life stage	Source	LH d.f.	LH MS	LH *F*	WR d.f.	WR MS	WR *F*	IC d.f.	IC MS	IC *F*
Seeds per fertilized ovule	Treatment	1	0·199	2·625	1	3·425	46·421**	1	1·001	5·306*
	Maternal plant	12	0·084	1·077	12	0·085	1·102	9	0·203	0·998
	Treatment × maternal plant	12	0·078	1·398	12	0·078	1·351	9	0·204	1·888
Rate of germination	Treatment	1	0·064	1·469	1	0·000	0·000	1	0·003	0·202
	Maternal plant	9	0·023	0·530	9	0·004	0·800	9	0·138	8·738**
Survival to 2 weeks	Treatment	1	0·002	1·998	1	0·001	0·362	1	0·013	5·485*
	Maternal plant	9	0·002	2·039	9	0·001	0·579	9	0·004	1·854
Survival from 2 weeks to flowering	Treatment	1	0·001	1·000	1	0·000	0·015	1	0·045	5·540*
	Maternal plant	9	0·001	0·667	9	0·016	0·704	9	0·011	1·405
Above-ground biomass	Treatment	1	10·43	4·357	1	18·15	7·924*	1	2·88	1·690
	Maternal plant	9	3·06	1·277	9	11·21	4·881*	9	4·06	2·246
	Treatment × maternal plant	9	2·39	3·563**	9	2·30	2·817**	9	1·81	2·042*

* $P < 0·05$, ** $P < 0·01$.

Lack of replication within maternal plants precludes a test of the treatment × maternal plant interaction for germination and survival variables.

TABLE 3. *Analysis of variance of the effects of population (fixed), treatment (self- versus cross-fertilization, fixed) and maternal plant (random) nested within population*

Life stage	Source of variation	d.f.	MS	*F*	*P*
Seeds per fertilized ovule	Treatment	1	3·66	34·71	0·000
	Population	2	2·93	26·69	0·000
	Maternal plant (population)	33	0·12	1·04	0·451
	Treatment × population	2	0·55	5·19	0·010
	Treatment × maternal plant (population)	33	0·11	1·61	0·026
Rate of germination	Treatment	1	0·032	1·48	0·235
	Population	2	4·304	77·77	0·000
	Maternal plant (population)	27	0·055	2·55	0·009
	Treatment × population	2	0·018	0·81	0·454
Survival to 2 weeks	Treatment	1	0·009	1·50	0·231
	Population	2	0·058	9·34	0·001
	Maternal plant (population)	27	0·009	1·39	0·197
	Treatment × population	2	0·021	3·36	0·050
Survival from 2 weeks to flowering	Treatment	1	0·005	1·546	0·224
	Population	2	0·005	1·850	0·177
	Maternal plant (population)	27	0·003	0·868	0·643
	Treatment × population	2	0·004	1·238	0·306
Above-ground biomass	Treatment	1	27·02	12·88	0·001
	Population	2	80·91	13·74	0·000
	Maternal plant (population)	27	6·108	2·821	0·004
	Treatment × population	2	1·222	0·579	0·567
	Treatment × maternal plant (population)	27	2·166	2·754	0·000

Lack of replication within maternal plants precludes a test of the treatment × maternal plant interaction for germination and survival variables.

Timing of self-compatibility in maternal plants

Pollen tube assays indicated that all maternal plants in populations IC and WR were transiently SI, i.e. stigmas were SI on the first day (self-pollen produced fewer than five pollen tubes) and became SC 24–48 h later. In the LH population, five plants were fully SC in first-day flowers (self-pollen produced >40 pollen tubes) and four were transiently SI. One plant was not assayed for pollen tube growth.

In the LH population, the relationship between inbreeding depression and the timing of SC in maternal plants was investigated. Using analysis of variance with SC type (early SC versus transient SI) and pollination treatment as fixed factors and maternal plants nested within SC type, no significant interactions were found

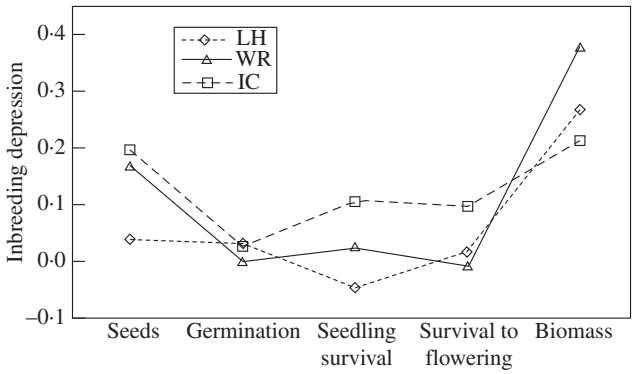

FIG. 2. Inbreeding depression at five life stages in three populations. Inbreeding depression was calculated as $1 - (w_s/w_o)$ using population mean fitness values. Seeds = the proportion of fertilized ovules developing into mature seeds; Seedling survival = the proportion of seedlings surviving to two weeks; Survival to flowering = the proportion of plants surviving from 2 weeks to flowering. Biomass indicates dry above-ground biomass at senescence.

TABLE 4. *Mean (standard deviation) performance of selfed and outcrossed progeny in three populations (LH, WR and IC)*

	LH			WR			IC		
	Selfed	Outcrossed	δ	Selfed	Outcrossed	δ	Selfed	Outcrossed	δ
Seeds per fertilized ovule	0·917 (0·098)	0·954 (0·057)	0·039	0·781 (0·100)	0·940 (0·858)	0·169	0·652 (0·231)	0·810 (0·163)	**0·195**
Proportion germinating	0·963 (0·056)	0·992 (0·193)	0·029	0·998 (0·008)	0·998 (0·008)	0	0·617 (0·193)	0·633 (0·216)	0·025
Proportion surviving to 2 weeks	0·859 (0·066)	0·820 (0·088)	–0·048	0·900 (0·076)	0·924 (0·077)	0·026	0·762 (0·098)	0·852 (0·105)	**0·106**
Proportion surviving from 2 weeks to flowering	0·975 (0·040)	0·992 (0·026)	0·017	0·933 (0·146)	0·925 (0·133)	-0·009	0·888 (0·129)	0·983 (0·053)	**0·097**
Above-ground biomass (g)	0·278 (0·268)	0·380 (0·242)	0·268	0·123 (0·172)	0·199 (0·204)	**0·382**	0·122 (0·138)	0·155 (0·186)	0·213
Ovules per flower				14·574 (1·342)	14·812 (1·296)	0·016			
Large pollen grains per flower				1248·618 (652·048)	1466·954 (844·728)	0·149			

δ (inbreeding depression) = $1 - (w_s/w_o)$.
δ values in boldface indicate those life stages for which selfed and outcrossed progeny were significantly different at $P < 0·05$.

TABLE 5. *Analysis of variance of the effects of treatment (self- versus cross-fertilization, fixed) and maternal plant (random) on pollen and ovule variables*

Response variable	Source of variation	d.f.	MS	*F*	*P*
Number of ovules per flower	Treatment	1	0.024	2.246	0.168
	Maternal plant	9	0.019	1.751	0.208
	Treatment × maternal plant	9	0.011	3.567	0.001
Number of large pollen grains per flower	Treatment	1	1.619	2.059	0.182
	Maternal plant	9	0.333	0.411	0.899
	Treatment × maternal plant	9	0.809	1.684	0.096
Proportion of small pollen grains	Treatment	1	6.368	2.172	0.170
	Maternal plant	9	8.390	2.892	0.065
	Treatment × maternal plant	9	2.901	0.864	0.558

between SC type and pollination treatment for any life stage (d.f. = 1, proportion of fertilized ovules developing to seeds: $F = 3·881$, $P = 0·089$; germination: $F = 0·715$, $P = 0·412$, survival to 2 weeks: $F = 0·066$, $P = 0·881$; survival from 2 weeks to flowering: $F = 0·015$, $P = 0·903$; biomass: $F = 0·017$, $P = 0·899$).

DISCUSSION

Self-incompatibility versus inbreeding depression for seed set

The results of this study indicate that both pre- and post-fertilization processes can limit self-seed set in some populations of *L. jepsonii*. In previous greenhouse studies, outcrossed seed set was substantially higher than selfed seed set in some populations (Goodwillie and Ness, 2005), but seed counts alone did not allow a distinction to be made between SI and early inbreeding depression. Fruit dissection data presented here indicate that the extent of both processes vary among populations. Population IC, which has a high frequency of plants with flowers that are SI for 2 d or more (Table 1), showed a marginally significant increase in unfertilized ovules with self-pollination. In contrast, the proportion of unfertilized ovules was low and not affected by cross-treatment in the two populations with earlier onset of SC. Self-seed set was significantly reduced by the abortion of fertilized ovules only in the two more outcrossing populations, WR and IC, which is interpreted as early inbreeding depression. Alternatively, seed abortion could be caused by late-acting SI (Seavey and Bawa, 1986), a phenomenon

that has been reported for distantly related taxa in the Polemoniaceae (LaDoux, 2004). However, all evidence to date indicates that the SI response in *Leptosiphon* occurs at the surface of the stigma and prior to fertilization (Goodwillie, 1997; Goodwillie *et al.*, 2004), suggesting that self-seed abortion is caused by inbreeding depression.

The evolution of inbreeding depression

The results of this study are somewhat congruent with the hypothesis that inbreeding depression is purged with self-fertilization. Cumulative inbreeding depression across five life stages was substantially lower in the LH population ($\delta = 0{\cdot}297$) than in the intermediate and highly outcrossing populations, for which cumulative inbreeding depression was similar ($\delta = 0{\cdot}495$ and $0{\cdot}501$). Consistent with theory on the evolution of inbreeding depression (Husband and Schemske, 1996), the magnitude of inbreeding depression differed significantly among populations only for early life stages (the proportion of fertilized ovules that produced seeds and seedling survival to 2 weeks), with the most outcrossing population showing the highest inbreeding depression. Deleterious recessive alleles of large effect are likely to be expressed early in life and are expected to be purged rapidly. In contrast, alleles that are mildly deleterious and mildly recessive are not purged readily with selfing and may be expressed throughout the lifetime. As a result, theory suggests that most of the inbreeding depression remaining in highly selfing populations will be expressed at later life stages, a pattern that was found in the present study and in 14 of 18 selfing species surveyed by Husband and Schemske (1996). However, although purging is expected to be less effective at later life stages, the trend toward higher inbreeding depression for biomass in the highly selfing population appears to be inconsistent with the purging hypothesis. In light of large variance in the biomass data and lack of significance of the difference among populations, however, it is difficult to assess whether this unexpected finding warrants an alternative evolutionary explanation.

Lack of congruence with previous inbreeding depression estimates for L. jepsonii

The present estimates of inbreeding depression were considerably higher than those from an earlier greenhouse study of *L. jepsonii*, in which cumulative inbreeding depression was below $0{\cdot}23$ in three populations (Goodwillie, 2000). For WR, the only population included in both studies, cumulative inbreeding depression was found to be $0{\cdot}166$ in the 2000 study, approx. one-third of the current estimate for the same life stages. Growth conditions appear to have been more stressful in the current study, and stress has been hypothesized to magnify the expression of inbreeding depression (Dudash, 1990). For outcrossed progeny in the WR population, survival rates and mean above-ground biomass of outcrossed progeny were substantially lower than in the previous experiment. Survival and growth in the current study appeared to be reduced by fungus gnats. In addition, plants were top-watered in the current study and experienced occasional drought stress, whereas plants in the 2000 study were continuously subirrigated.

Whether or not stress is the source of the difference, however, the present studies underscore the potential effects of environmental variability on inbreeding depression and, therefore, the difficulties in arriving at meaningful estimates (Hayes *et al.*, 2005). Which of the estimates are more representative of natural populations? The fungus gnat infestation is probably not typical of field populations, which experience little insect herbivory (C. Goodwillie, personal observation). On the other hand, mean above-ground biomass of field-grown plants at the WR population ($0{\cdot}082$ g, $n = 30$) is even lower than that observed in the current study ($0{\cdot}199$ g), suggesting that resources are strongly limited under natural conditions. Thus, on the whole, the current higher estimates of inbreeding depression may be closer to the levels experienced in the field.

Inbreeding depression and the evolution of mating systems

As discussed above, the magnitude of inbreeding depression may be a consequence of the mating-system history of a population. In turn, inbreeding depression is expected to affect current selection on the selfing rate. With few exceptions (e.g. Holsinger, 1991), models of mating-system evolution include inbreeding depression as the key selective parameter that favours outcrossing. Inbreeding depression from this study for the IC and WR populations are near the $0{\cdot}5$ threshold necessary to oppose the genetic transmission advantage of selfing and maintain outcrossing. In contrast, cumulative inbreeding depression is well below that value in the LH population, suggesting that selection may favour higher selfing rates in that population.

While many models of plant mating-system evolution treat inbreeding depression as a population parameter, theory indicates that associations between mating-system modifiers and fitness loci can have important effects in the selection of mating systems (Holsinger, 1988; Uyenoyama and Waller, 1991). Such associations are predicted to yield a negative relationship among lineages in traits that promote selfing and the magnitude of inbreeding depression. For this reason, family level inbreeding depression values may be more relevant to mating-system dynamics than population estimates, which has motivated a number of empirical studies of family level variation (e.g. Carr *et al*., 1997; Mutikainen and Delph, 1998). In *L. jepsonii*, it was found that inbreeding depression varied among maternal families for some life stages (Tables 2 and 3), including ovule number, for which population level inbreeding depression was not significant. However, family-level inbreeding depression was unrelated to the timing of SC in maternal plants of the LH population, a trait that is likely to affect the selfing rate (Fig. 3). Moreover, the relative magnitude of inbreeding depression was not consistent across different life stages (Fig. 3).

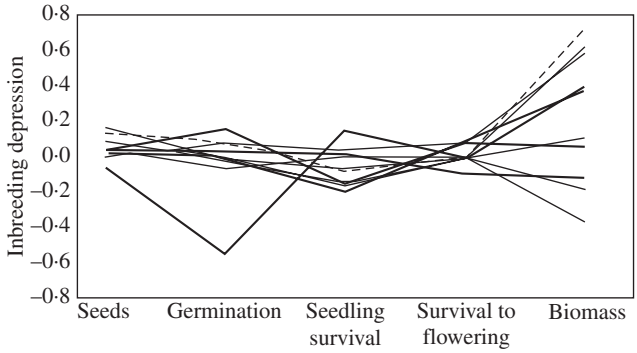

FIG. 3. Inbreeding depression at five life stages for ten maternal families in the LH population. Bold lines indicate families derived from maternal plants with early SC. Light lines indicate families from maternal plants with delayed SC. Dotted line indicates a family for which the maternal plant SC status is unknown. Inbreeding depression was calculated as $1 - (w_s/w_o)$ using mean fitness values for maternal plants. See Fig. 2 caption for explanation of life stages.

Thus, the present limited data do not provide evidence that associations between selfing rate modifiers and fitness loci are important in the evolutionary dynamics of the mating system in this population. While some empirical studies have found a negative relationship among families between inbreeding depression and traits that promote selfing (Chang and Rausher, 1999; Vogler *et al*., 1999; Takebayashi and Delph, 2000), the majority have not, indicating that purging does not occur consistently at the level of lineages within populations (reviewed in Byers and Waller, 1999)

Evolutionary stability of mixed mating in L. jepsonii

Populations of *L. jepsonii* undergo mixed mating at two levels. In all populations studied, some individuals exhibit transient SI, a phenomenon that is expected to confer mixed mating whenever pollinator visitation is variable or intermediate. Moreover, all populations contain variation in the strength and timing of SI, such that some individuals appear to be largely selfing and others more outcrossing. Is this mixed mating system evolutionarily stable and, if so, what processes maintain it?

No evidence that the relative magnitude of male and female inbreeding depression stabilizes mixed mating in the WR population of *L. jepsonii* was found. According to the model of Rausher and Chang (1999), mixed mating can be evolutionarily stable when the average of male and female inbreeding depression is <0·5 and female inbreeding depression is >0·5. In contrast, in the present study, it was found that the mean of male and female inbreeding depression was above 0·5 (δ = 0·537) and inbreeding depression was greater in male than in female expression. The present study did not include all components of male and female fitness. A complete estimate of female inbreeding depression would include a measure of mature seed set in selfed and outcrossed progeny. An inclusive measure of male inbreeding depression might include traits that affect pollinator visitation rate, such as corolla size and pollinator rewards, in addition to pollen production (Rausher and Chang, 1999). Previous results show minor but significant inbreeding depression for flower size in the WR population (δ = 0·012, C. Goodwillie unpublished data), so inbreeding depression for male fitness in this study may be somewhat underestimated. However, these additional components of fitness are likely to increase the mean level of inbreeding depression, a value already higher than that required for stability in the model of Rausher and Chang (1999). It is noted, however, that the multiplicative cumulative estimates include life stages for which inbreeding depression is not significant. For example, higher male than female inbreeding depression results from substantial, though nonsignificant, differences in mean pollen production in selfed and outcrossed progeny. Thus the present findings should be considered with some caution. Nevertheless, they do contribute to the very limited empirical literature on the relative magnitude of male and female inbreeding depression. In *Mimulus guttatus*, greater inbreeding depression has been observed in male than in female function (Carr and Dudash, 1995). In contrast, *Phacelia dubia* (del Castillo, 1998) and *Ipomoea purpurea* (Chang and Rausher, 1999) show greater lifetime inbreeding depression for female than male components of fitness, although their magnitudes do not fall within the range for which stable mixed mating is predicted by Rausher and Chang (1999).

Further insights on the mating system of *L. jepsonii* may come from investigations of pollen and seed discounting, reproductive assurance and temporal variation in pollinator visitation, factors that have been theorized to play a role in the maintenance of mixed mating (Johnston, 1998; Morgan and Wilson, 2005). Although the frequent occurrence of partial SI and its adaptive value have been emphasized increasingly (Levin, 1996; Stephenson *et al*., 2000), the evolutionary stability of partial SI has received relatively little attention. The number of self-incompatibility alleles in a population may be a critical determinant of the outcome of selection on modifiers of SI (Vallejo-Marin and Uyenoyama, 2004). A full understanding of the evolutionary dynamics of SI variation in *L. jepsonii* may require consideration of these factors, in addition to more general parameters such as inbreeding depression.

Conclusions

The cumulative evidence from comparative studies of plant taxa suggests that purging is an inconsistent force in the evolution of mating systems (Byers and Waller, 1999). However, the present study of intraspecific variation is largely congruent with the previous species comparison in *Leptosiphon* (Goodwillie, 2000) and provides further evidence that purging does occur in this plant taxon and may play an important role in the evolution of its diverse mating systems.

ACKNOWLEDGEMENTS

We thank S. Gardiner, B. Neel, K. Patel, S. Ross, S. Spencer and J. Weber for assistance with plant cultivation and C. Jolls for generously sharing growth chamber facilities. This work was supported by National Science Foundation grant DEB-0212903 to C.G.

LITERATURE CITED

Affre L, Thompson JD. 1999. Variation in self-fertility, inbreeding depression and levels of inbreeding in four *Cyclamen* species. *Journal of Evolutionary Biology* **12**: 113–122.

Busch JW. 2005. Inbreeding depression in self-incompatible and self-compatible populations of *Leavenworthia alabamica*. *Heredity* **94**: 159–165.

Byers DL, Waller DM. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics* **30**: 479–513.

Carr DE, Dudash MR. 1995. Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. *Heredity* **75**: 437–445.

Carr DE, Dudash MR. 1996. Inbreeding depression in two species of *Mimulus* (Scrophulariaceae) with contrasting mating systems. *American Journal of Botany* **83**: 586–593.

Carr DE, Fenster CB, Dudash MR. 1997. The relationship between mating system characters and inbreeding depression in *Mimulus guttatus*. *Evolution* **51**: 363–372.

del Castillo RA. 1998. Fitness consequences of maternal and nonmaternal components of inbreeding in the gynodioecious *Phacelia dubia*. *Evolution* **52**: 44–60.

Chang SM, Rausher MD. 1999. The role of inbreeding depression in maintaining the mixed mating system of the common morning glory, *Ipomoea purpurea*. *Evolution* **53**: 1366–1376.

Charlesworth D, Charlesworth B. 1979. The evolutionary genetics of sexual systems in flowering plants. *Proceedings of the Royal Society of London, Series B* **205**: 513–530.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**: 237–268.

Cheptou PO, Berger A, Blanchard A, Collin C, Escarre J. 2000. The effect of drought stress on inbreeding depression in four populations of the Mediterranean outcrossing plant *Crepis sancta* (Asteraceae). *Heredity* **85**: 294–302.

Crnokrak P, Barrett SCH. 2002. Perspective. Purging the genetic load: a review of the experimental evidence. *Evolution* **56**: 2347–2358.

Darwin CR. 1877. *Different forms of flowers on plants of the same species*, Reprinted 1986. Chicago: University of Chicago Press.

Dudash MR. 1990. Relative fitness of selfed and outcrossed progeny in a SC, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* **44**: 1129–1139.

Fisher RA. 1941. Average excess and average effect of a gene substitution. *Annals of Eugenics* **11**: 53–63.

Fishman L. 2001. Inbreeding depression in two populations of *Arenaria uniflora* (Carophyllaceae) with contrasting mating systems. *Heredity* **86**: 184–194.

Goodwillie C. 1997. The genetic control of self-incompatibility in *Linanthus parviflorus* (Polemoniaceae). *Heredity* **79**: 424–432.

Goodwillie C. 1999. Multiple origins of self-compatibility in *Linanthus* section *Leptosiphon*: phylogenetic evidence from internal-transcribed-spacer sequence data. *Evolution* **53**: 1387–1395.

Goodwillie C. 2000. Inbreeding depression and mating systems in two species of *Linanthus* (Polemoniaceae). *Heredity* **84**: 283–293.

Goodwillie C, Ness JM. 2005. Correlated evolution in floral morphology and the timing of self-compatibility in *Leptosiphon jepsonii*. *International Journal of Plant Sciences* **166**: 741–751.

Goodwillie C, Partis KL, West JW. 2004. Transient SI confers delayed selfing in *Leptosiphon jepsonii* (Polemoniaceae). *International Journal of Plant Sciences* **165**: 387–394.

Goodwillie C, Kalisz S, Eckert CG. 2005. The evolutionary enigma of mixed mating in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Evolution, Ecology and Systematics* **36**: 47–79.

Grant V, Grant KA. 1965. *Flower pollination in the phlox family.* New York, NY: Columbia University Press.

Hayes CN, Winsor JA, Stephenson AG. 2005. Environmental variation influences the magnitude of inbreeding depression in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *Journal of Evolutionary Biology* **18**: 147–155.

Holsinger KE. 1988. Inbreeding depression doesn't matter: the genetic basis of mating system evolution. *Evolution* **42**: 1235–1244.

Holsinger KE. 1991. Mass action models of plant mating systems: the evolutionary stability of mixed mating systems. *American Naturalist* **138**: 606–622.

Holsinger KE, Feldman, Christiansen FB. 1984. The evolution of self-fertilization in plants: a population genetic model. *American Naturalist* **124**: 446–453.

Holtsford TP, Ellstrand NC. 1990. Inbreeding effects in *Clarkia temblo-riensis* (Onagraceae) populations with different natural outcrossing rates. *Evolution* **44**: 2031–2046.

Husband BC, Schemske DW. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.

Johnston MO. 1998. Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. *Genetica* **102/3**: 267–278.

Johnston MO, Schoen DJ. 1994. On the measurement of inbreeding depression. *Evolution* **48**: 1735–1741.

Johnston MO, Schoen DJ. 1996. Correlated evolution of self-fertilization and inbreeding depression: an experimental study of nine populations of *Amsinckia* (Boraginaceae). *Evolution* **50**: 1478–1491.

Kalisz S, Vogler DW. 2003. Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* **84**: 2928–2942.

Kelly JK, Rasch A, Kalisz S. 2002. A method to estimate pollen viability from pollen size variation. *American Journal of Botany* **89**: 1021–1023.

LaDoux T. 2004. *Self-incompatibility in Polemoniaceae*. PhD Thesis, Claremont Graduate University, USA.

Lande R, Schemske DW. 1985. The evolution of inbreeding depression and self-fertilization in plants. I. Genetic models. *Evolution* **39**: 24–40.

Levin DA. 1989. Inbreeding depression in partially self-fertilizing phlox. *Evolution* **43**: 1417–1423.

Levin DA. 1996. The evolutionary significance of pseudo-self-fertility. *American Naturalist* **148**: 321–332.

Lloyd DG. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *American Naturalist* **113**: 67–79.

Lloyd DG. 1992. Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences* **153**: 370–380.

Morgan MT, Wilson WG. 2005. Self-fertilization and the escape from pollen limitation in variable pollination environments. *Evolution* **59**: 1143–1148.

Mustajarvi K, Siikamaki P, Akerberg A. 2005. Inbreeding depression in perennial *Lychnis viscaria* (Caryophyllaceae): effects of population mating history and nutrient availability. *American Journal of Botany* **92**: 1853–1861.

Mutikainen P, Delph LF. 1998. Inbreeding depression in gynodioecious *Lobelia siphilitica*: among-family differences override between-morph differences. *Evolution* **52**: 1572–1582.

de Nettancourt D. 1977. *Incompatibility in angiosperms*. Berlin: Springer-Verlag.

Rausher MD, Chang SM. 1999. Stabilization of mixed-mating systems by differences in the magnitude of inbreeding depression for

male and female fitness components. *American Naturalist* **154**: 242–248.

Richards AJ. 1997. *Plant breeding systems*, 2nd edn. London: G. Allen and Unwin.

Schemske DW, Goodwillie C. 1996. Morphological and reproductive characteristics of *Linanthus jepsonii* (Polemoniaceae), a newly described, geographically restricted species from northern California. *Madrono* **43**: 453–463.

Seavey SR, Bawa KS. 1986. Late-acting self-incompatibility. *Botanical Review* **52**: 196–217.

SPSS. 2005. *SPSS version 13·0.* Chicago: SPSS, Inc.

Stephenson AG, Winsor JA, Richardson TE, Singh A, Kao T-H. 1992. Effects of style age on the performance of self and cross pollen in *Campanula rapunculoides*. In: Ottaviano E, Mulcahy DL, Sari Gorla M, Bergamini Mulcahy G, eds. *Angiosperm pollen and ovules*. New York, NY: Springer, 77–121.

Stephenson AG, Good SV, Vogler DW. 2000. Interrelationships among inbreeding depression, plasticity in the self-incompatibility system, and the breeding system of *Campanula rapunculoides* L. (Campanulaceae). *Annals of Botany* **85**: 211–219.

Takebayashi N, Delph LF. 2000. An association between a floral trait and inbreeding depression. *Evolution* **54**: 840–846.

Uyenoyama MK, Waller DM. 1991. Coevolution of self fertilization and inbreeding depression. I. Mutation-selection balance at one and two loci. *Theoretical Population Biology* **40**: 14–46.

Vallejo-Marin M, Uyenoyama MK. 2004. On the evolutionary costs of self-incompatibility: incomplete reproductive compensation due to pollen limitation. *Evolution* **58**: 1924–1935.

Vogler DW, Stephenson. 2001. The potential for mixed mating in a self-incompatible plant. *International Journal of Plant Sciences* **162**: 801–805.

Vogler DW, Filmore K, Stephenson AG. 1999. Inbreeding depression in *Campanula rapunculoides* L. I. A comparison of inbreeding depression in plants derived from strong and weak self-incompatibility phenotypes. *Journal of Evolutionary Biology* **12**: 483–494.